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TRANSLATION

CHEMISTRY OF BLUE-GREEN
ALGAE (CYANOPHYCEAE)

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Chemistry of Blue-Green Algae (Cyanophyceae)

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G. K. Barashkov

Bluish-green algae are distributed preferably in fresh water. During some periods of the year the sea form of these algae may constitute a considerable part of the phytoplankton, but their total value in the sea is inferior to the role of other types of algae.

In recent years are appearing quite large numbers of investigations dealing in photosynthesis and mineral nutrition of bluish-green algae, as well as dealing in other problems of their physiology. In these conditions of great importance is the knowledge of their chemical composition as bases to comprehend the processes occurring in them. The knowledge of the chemistry of algae is also necessary for proper evaluation of their role in nature. The existing review of the biochemistry and physiology of bluish-green algae (Fogg 1956) has become somewhat obsolete, besides, no sufficient attention is devoted in it to the chemistry of these organisms.

Carbohydrates

The total amount of carbohydrates in bluish-green algae reaches 70-75%, i.e. the very same order, as in brown and red algae (Goryunova, 1950).

Mono and Oligosaccharides. Conventional determination of reducing sugars in alcohol extracts of bluish-green algae with the aid of Feling liquid always gives a negative result. For example, S.V.Goryunova (1950) found no reducing sugars in Oscillatoria splendida, and (Payen, 1953) in Rivularia bullata, Calothrix pulvinata and Nostoc commune. But in some form have been found traces of free monoses. And so, with the aid of chromatographic analysis of alcohol extracts Nostoc muscorum, grown in an artificial medium from $C^{14}O_2$, was found glucose and in free form, and in form of

phosphate (Norris L. Norris .R.E; Calvin, 1955). It must be taken into consideration however that nostoc possesses a greater slimy cover, and this does not allow to obtain a bacterial less culture. It is therefore necessary to practice care with respect to this fact.

In oligosaccharides in bluish-green algae have been detected trehalose-sugar, found under natural conditions only in mushrooms and red algae. In alcohol extracts Nostoc muscorum were detected traces of saccharose (Norris, L; Norris R.E.Calvin, 1955) but this is not without doubt.

Polyoses. Up until now polysaccharides of bluish-green algae have not been sufficiently investigated. But a majority of scientists, occupying themselves with this problem, assumes, that the basic component of these algae are mucoid polysaccharides analogous to such of red algae (Fogg 1956). It should be pointed out, that since 1938 when Payen undertook to study slugs of three types of bluish-green algae, progress in explaining the structure of these polyoses was very small, partially because of their exclusive stability to acid and alkali hydrolysis. Kylin (1943) discovered no free sugars in sea algae Calothrix scopulorum.

Mucoid Polysaccharides of bluish-green algae are well extracted by hot water extraction. In this way was extracted a slug from Nostoc sp. (Hough, Jones, Wadman, 1952), from Phormidium tenue (Payen, 1933), from Rivularia sp. (Payen, 1938) and from calothrix (Kylin, 1943). From Anabaena cylindrica these polysaccharides were extracted with a 4%- sodium hydroxide (Bishop, Adams, Hughes, 1954); from Oscillatoria splendida - with hot 2%-hydrochloric acid (Goryunova, 1950); from Nostoc muscorum - 10%-trichloroacetic acid and then settled with 90%-ethanol (Biswas, 1957a). In the la ter case the yield equalled 3.5 - 4% of dry weight of algae.

In Hydrolysates of various algae are detected various sugars and uronic acids. When investigating polysaccharides of fresh water algae Phormidium tenue was obtained a dextro-rotary extract, in which after soft hydrolysis were found rhamnose, [illegible]

nose, glucose, and galactose. From neutralized hydrolysate was obtained a light deposition of mannuronic acid (Payen,1953). Previously,when hydrolyzing polysaccharides from Rivularia bullata, Calothrix pulvinata and Nostoc commune were obtained in addition to uronic acids glucose and arabinose in the first algae, galactose and mannose in the second and arabinose in the third one (Payen, 1938).

Mucoid polysaccharides from Calothrix scopulorum have in their composition ester-bound sulfuric acid, neutralized by calcium and magnesium (Kylin,1943). This analogy with polysaccharides of red algae supplements the difficulty of their hydrolyzing. And so, when hydrolyzing slugs of Nostoc it is quite easy to hydrolyse about 60%. The remaining part cleaves at a much lower rate. The poorly hydrolizable part consisted of uronic acids- glucouronic and galactouronic. In spite of the greater resistance of the polysaccharide to hydrolysis, it was possible to establish, that it contains about 30% of hexuronic acids, 10% rhamnose,25% xylose and 35% galactose with small admixture of glucose and unidentified sugar (Hough,Jones,Wadman,1952). Polysaccharides,extracted from Nostoc muscorum with trichloroacetic acid and 4%-solution of caustic soda, were found to be very similar in qualitative composition (Biswas,1957a). It was explained,that these polysaccharides, or polysaccharide, are in someway bound with nucleinic acids of the cells (Biswas,1956).

A highly resistant to hydrolysis polysaccharide was separated from Anabaena cylindrica. After continuous hydrolysis the researchers detected in it glucose, galactose, xylose,rhamnose, arabinose and glucouronic acid. The molar ratios were found to be 5:1:4:1:1:4 respectively (Bishop, Adams,Hughes,1954). The structure of the polysaccharide has not been investigated, it can only be assumed after continuous turning of the extracts, that mostly . in it are alpha-form bonds. Experiments with acetylation of this polysaccharide were found to be without results.

It is evident from above mantioned data that do not coincide with each other neither in quality nor in quantity. Most likely this is explained by the fact that in bluish-greean algae exist several hard to separate polyoses,differing in various

forms. The opinion was also expressed, that these polysaccharides can be found in several states, differing from each other in physical properties. These differences are caused by various degrees of deaggregation of molecules (Goryunova,1950).

As a general property of mucoid polysaccharides of bluish-green algae should be mentioned their poor hydrolyzability with acids and alkalis. The fact of incomplete hydrolysis of Oscillatoria amoena shells of polysaccharosis with lysocine also attests to the same (Fuhs,1958).

We can speak about the presence in bluish-green algae of a reserve polysaccharide - " starch of bluish-green ". They are detected by the reddish-brown color with iodine and, probably, are present in plants in form of submicroscopic granules (Kylin, 1943). Prior to Kylin's investigation this glycogeno kind substance has been detected also by other scientists (Hegler,1901, Buetschli,1902, Fischer,1905, Payen,1938).

Starch of blue-greens from Oscillatoria sp.was separated with cold water,in which it dissolved well. The solution had greater positive rotation (Hough,Jones,Wadman,1952). This "starch " hydrolyzed easily with a malt extract and mineral acids, but not with acetic. During fermentation hydrolysis the given polysaccharide,separated from Calothrix scopulorum, gave maltose and glucose (Kylin,1943). This allows to assume an alpha-form of the 1.4 type bond, in spite of the fact that this type may not be unique.

During the methylation of the mentioned polysaccharide, separated from Oscillatoria sp. it revealed a positive rotation. In hydrolysate with the aid of paper chromato graphy and a column were found trimethyl-,tetramethyl-and a small amount of dimethyl glucose (Hough,Jones,Wadman,1952). These data allow to assume,that per average number of glucose radicals, equalling 23-26, in the chain of the amylopectin type exists one nonreduced terminal group.

Molecules of somewhat different value give the synthesis of starch of bluish-green from glucoso-1-phosphate with the aid of ferments from Oscillatoria princeps. The synthesized polysaccharide contained 14-16 glucose radicals. Its remaining properties, such as solubility in cold water and coloring with iodine, coincided with the pro-

perties of natural starch from bluish-greens (Fredrick,1951, 1952, 1953).

In addition to slugs and starch,Kylin,1943 has occasionaly found in bluish-greens a substance, reacting as cellulose when treated with iodine and sulfuric acid. When studying the structure of the cellular shell of certain types of bluish-green algae with the aid of an electron microscope were found fibrils of a cellulose like substance (Schulz,1955; Drews,Niklowitz,1956, Niklowitz,Drews,1956).But thorough chemical analyses revealed practically no cellulose in Oscillatoria splendida (Goryunova 1950). Qualitative reactions in this substance (Metzner,1955) during the study of five types of bluish green algae were found to be negative in contrast to certain results of Kylin.

Quantitative reactions of carbohydrates of bluish-green algae are not personified, with the exception of the starch reaction. The cellular walls of certain algae as result or treating with periodate and Schiff reagent (leukofuchsin) are colored red (Pringsheim,1954). Such a color of the shell is obtained after treating the algae with a reagent on a pectine substance of higher plants - with red ruthenium. The methylene blue colors the cellular walls in blue color, reaching in some instances to bluish - violet. All these data, as it would appear, indicate the presence in the cellular walls of five investigated types of algae of pectin like substances (Metzner,1955). It is known however, that similar reactions give also other polysaccharides, with uronic acids in their composition. Consequently the results of microchemical qualitative determinations should not be considered as proof of the presence of these or any other polysaccharides in the algae.

The available data on the chemistry of carbohydrates allow to assume a greater affinity between polysaccharides of bluish-green and red algae.This is confirmed first of all by the presence of trehalose and esterification of polysaccharides with sulfuric acis. The very high stability of the carbohydrate complex of bluish-green algae even against highly reactive reagents also reminds the properties of polyoses of red

algae.

Nitrogen containing compounds

The amount of nitrous substances in bluish-green algae is quite high. The total content of nitrogen in Anabaena cylindrica culture of normal growth goes up to 6.51% of the dry weight of cells, and albumina was discovered in the amount of about 35% (Fowden,1951). In contrast to representatives of other types of algae,blue-green algae show no reverse connection between the accumulation of lipides and the content of nitrogen in the cells (Collyer, Fogg,1955). The content of nonalbuminous nitrogen in these cells may exceed the content of albuminous nitrogen. This has been observed by (Krishna-Pillai, 1956) in Oscillatoria, Spirulina, Aphanotheco and Phormidium tenue of salted lagoons of India.

From individual albumina from bluish-green algae are known chromoproteides- phycoerythrin and phycocyanines, which appear to be additional pigments in them . A study of phycobilines from Arthrospira maxima showed that it contains two phycocyanines - C-phycocyanine and allophycocyanine,differing from each other by the chromomorphous groups (O'h-Eocha,1958). In addition (Zastrow,1953) found in cells of certain types noticeable granules, consisting of reserve albumin of bilichromoproteide "Cyanophycin". The small nuclei of cyanophycin, among a number of other formations, in 18 forms - Anabaena Nostoc, Cylindrospermum, Scytonema, Tolypothrix, Oscillatoria and Lyngbya have also been observed by Tischer, 1957.

Albumina of bluish-green algae consist of ordinary amino acids, the amounts of which contained in some of them are described in table.

Amino acid composition of bluish-green algae (in % of the amount of albuminous nitrogen).

Amino acids	Anabaena cylindrica (Fowden, 1951)	Phormidium (Mazur, Clarke, 1938)	Nostoc muscorum (Magee, Burris, 1954)	Amino acids	Anabaena cylindrica (Fowden, 1951)	Phormidium (Mazur, Clarke, 1938)	Nostoc muscorum (Magee, Burris, 1954)
Aspartic	6.9	0.5	7.7	Arginine	11.7	9.2	19.7
Glycine	6.5	1.6	5.4	Histidine	2.5	3.8	2.6
Threonine	5.7	—	3.1	Lysine	6.6	0.0	5.3
Alanine	6.0	5.2	6.8	Tryptophan	1.0	0.2	—
Tyrosine	1.6	1.8	2.1	Methionine	1.2	2.0	0.4

table continued

Valine	7.0	6.7	3.3	Cystine	—	0.0	1.4
Phenylalanine	2.9	11	2.4	Amides	8.0	7.8	7.7
Serine	2.4	—	4.2	Humines	—	2.7	—
Glutamic	5.6	4.4	6.2	Oxyproline	—	2.3	—
Leucine	6.2	2.1	4.6	Bases	—	—	5.4
Isoleucin	3.9	—	2.8	total nitrogen	89.7	76.1	93.3
Proline	5.0	7.0	2.5				

It is evident from this table, that in the investigated algae are encountered relatively large quantities of arginine and amides. Arginine is detected also in the composition of the central body and cytoplasma of certain blue-green algae and by the positive histochemical reactions (Biswas,1957b).

Of other amino acids could be mentioned the presence in hydrolysates of Anabaena cylindrica, Oscillatoria, Microcoleus vaginatus and Mastigocladus laminosus a.E-diaminopimelic acid; its amount constitutes 0.1 - 0.8% of the dry weight (Work,Dewey,1953). In his review (Fogg,1956) underlined, that this acid was found also only in some bacteria and in green algae Chlorella ellipsoidea.

After incubation of Nostoc cells with sodium bicarbonate,containing traced carbon C^{14} within a period of 5 minutes and studying the alcohol extract from it was obtained active citrulline. The basic part of the activity, up to 70%,belonged to the carbamyl group ($NH-CO-NH_2$) and only 2-3% went to the carboxyl group (Linko,Holm-Hanson,Basham, Calvin,1957). Carbamyl groups goe next, most likely,for the synthesis of nucleotides.

Nucleoproteides, of bluish-green algae are no differnt in quality composition from nucleoproteides of other organisms (Biswas,1956) Mockeridge,1927, was first to find adenine,guanine,cytosine and uranyl in Nostoc hydrolysates and he identified same. Then Biswas,1951 by a pyronine and methyl green dye came to the conclusion about the accumulation in the central part of cells of these algae of ribonucleonic and desoxy ribonucleonic acids. Magee, Burris,1954, found in Nostoc muscorum adenine, cytosine, guanine,thymine, uranyl and xanthine, the amounts of which were 0.4, 1.1, 0.3, 0.3, 1.0 and 2.3% respectively of the total nitrogen in the hydrolysate. It should be pointed out, that qualitative reactions can also here lead to wrong conclusions. And so,

cells of Oscillatoria princeps gave no positive Feylgen reactions-characteristic reaction for nuclear substance. Apparently, this can be explained by the presence in algae cells of certain albumina, suppressing the Feylgen reaction.

It was found, that in DNK (Desoxyribose nucleic acid) the ratio of purines to pyrimidines equals 0.79 (Shinke, Ishida, Ueda, 1957). This ratio does not appear to be constant for various bluish-green algae. For example, in DNK of fresh water type - Anacystis nidulans-it was found to be equal 1.04 (Low, 1958).

Pteridines have been detected chromatographically in Anacystis nidulans, Anabaena variabilis and Nostoc muscorum in amount, equalling 0.1-0.2% of dry weight of algae (Forrest, Van Baalen, Myers, 1957), and from A. Nidulans was also separated alpha-glucoside of biopterin (Forrest, Van Baalen, Myers, 1958).

But the question concerning the presence of pteridines in living cells of algae remains unanswered, because a thorough study of the condition of formation of the yellow fluorescent compound on chromatograms showed, that it appears to be an artefact of chromatography on paper (Fuller, Anderson, Nathan, 1958).

It has been noticed already long ago that in waters blooming with bluish-green algae (in reservoirs), together with a reduction in the number of nitrate and ammonium nitrate, appears and accumulates with time organic nitrogen, mainly in form of amino acids (Aleyev, Mudretsova, 1937). The fixing elementary nitrogen can be separated in form of peptides as well as amides. This was shown on an Anabaena cylindrica culture. It was explained that the separated substances are not specific, for bluish-green algae, nitrogen fixation products. They are necessary for normal growth, and their separation takes place always, when the algae grow under normal conditions. This separation does not depend upon the volume of culture media, presence of accessible bound nitrogen and glucose, as well as upon the difference in light intensity (Fogg, 1952). The ability of bluish-green algae to fix atmospheric nitrogen is being used practically in China. Into water, which cover rice fields, are added

certain cultures of actively binding nitrogen of bluish-green algae to fertilize the rice with nitrous compounds.

As already mentioned, bluish-green algae by the qualitative amino acid composition and the composition of nucleinic acids are no different from other algae. Consequently efforts are made, on the basis of data pertaining to amino acid composition of algae, to compare same to plants, offering most valuable nutritive albumina for farm animals (Kclousek, Zazvorka,1957).But the mechanical use of results of chemical analyses to evaluate the nutritive value of bluish-green algae without consideration of other factors may lead to a greater error. And so, the introduction of freshly collected cells Microcystis aeruginosa into the abdominal cavities of white mice caused panting and death of the animals within 4-48 hours (Hughes,Gorham,Zehnder,1958).

Lipides

The content of lipides in bluish-green algae varies within small limits. In Anabaena cylindrica and Oscillatoria cultures the lipides amounted to 2 - 12% of dry weight (Collyer, Fogg,1955) .

Fats in Gleeotrichia echinulata consist in approximate 60% of unsaturated fatty acids, i.e.they are analogous in this respect with lipides of other types of algae (Mazur,Clarke,1942). In addition, Mazur and Clarke noticed the presence in that algae of alcohols and hydrocarbons. Goodwin and Taha,1951, detected in Oscillatoria sp. unidentified steroides. On the other hand Carter and co-workers found no sterols in the investigated algae (Carter,Heilbron,Lythgoe,1939).

Pigments of bluish green algae have been quite thoroughly investigated. In the review, devoted to pigments (Cook,1945) noticed the presence in them of chlorophyll a, beta-carotin and several unindentified carotinoides. In addition to chlorophyll a Handke,1954-1955 in alcohol extracts of 10 types of algae, obtained in pure cultures, detected chlorophyll b. Chlorophyll b was also found in algaes of hot sources Yellowstone Park, California, Nevada (Inman,1940). Still Fogg does not consider irrefutible

the presence of chlorophyll b in bluish-green algae (Fogg,1956).

Of the carotinoids, in addition to beta-carotin, were found small amounts of alpha-carotin, myxoxanthene, myxoxanthophyll and oscillaxanthine (Karrer, Jucker, 1950). The presence in bluish-green algae of myxixanthine is highly specific and can serve as an indication of their development, if in the past this substance is found during the study of depositions in this or any other reservoir (Vallentyne,1954). Among all carotinoids per fraction of carotin 30-60% belong in Anabaena cylindrica, A.variabilis, Coccochloris elabens, Cylindrospermum sp, Mastigocladus laminosus, Microcoleus vaginatus and Nostoc muscorum algae. Other important components of carotinoids in these algae are echinenone, myxoxanthophyll and zeaxanthin. Lutein has not been found in the enumerated algae (Goodwin,1957; Tischer,1958; Lefrancois,1960).

In this way, by the composition and ratio of carotinoids bluish-green algae differ from other photosynthesizing organisms. It is necessary to mention, that the ratio of carotinoids differs in various forms. But when studying the different quality of pigments in various types of bluish-green algae it is necessary obligatorily to consider the conditions of their existence. For example, at higer temperatures as compared with normal in Oscillatoria subbrevis was always observed a higher content of carotinoids regardless of the illumination intensity (Garnier,1958). The very same thing has been observed in Anacystis nidulans and Anabaena sp. (Halldal,1958).

Exactly like red algae, bluish green algae contain bilichromoproteins, or phycobilines, physoerythrin, phycocyanin, allophycocyanines and others (Guerin-Duniartrait, 1960).

Other Investigations

Ash content in bluish-green algae may reach up to 10-20%, increasing in algae, thriving in more saline waters. The basic part of the ash is made up of ferric sulfate, magnesium sulfate, calcium and potassium sulfates. The greater the amount of chlorine ions in the surrounding water the greater is the sulfate ion accumulation in the algae cells. In the ash were also detected J,B,Zn,Cu,Na,Cl (Krishna-Pillai,1956).

Ferments of bluish-green algae have been only partially investigated. Gupta,1953, noticed that in the surrounding medium they do not form. In the cells was found lipase, catalase, proteinase, whereby the catalase of heat resistant Oscillatoria sp. has reverse properties (Kubin,1959). Not detected were amylases, inulase, raffinase, oxidase, peroxidase, cellulase, pectinase and glycolytic ferments, the presence of invortase is doubtful. The absence of basic ferments, inherent of starch accumulating higher plants (amylase invertase), compels to assume, that the name "starch" attributed to one of the polysaccharides of bluish-green algae, appears to be conditional. It is therefore advisable to call it "starch of bluish-green". Interesting is the absence in Anacystis nidulans of aldolase (Richter,1959). This indicates the extraordinary way of formation of hexoses during photosynthesis.

A whole series of experiments were carried out by Fredrick,1954, 1956, 1957, 1959 for the purpose of studying ferments, synthesizing polyglucoside in Oscillatoria princeps. He discovered the participation of two ferments in this process - phosphorylases and branching ferment, whereby the activity of the latter was approximately 20 times greater than phosphorylases. The reaction mechanism of the branching ferment lies in the fact, that the glucose radical of the glucoso-l-phosphate attaches itself to the 6-th carbon atom of the dextran radical with the formation of a-1,6-glucoside bond (Fredrick, Mulligan, 1955). Certain differences between the polyglucoside composition of the mutant and ordinary roots of this algae (Fredrick,1956) is explained by the differences in concentrations of branching ferment and the presence in normal root of phosphorylases-inhibiting the chenant agent of amino acid nature (1959). From the properties of both ferments can be mentioned the sharp inhibition of same by ionic surface-active substances and metal ions. This inhibition is removed upon the addition of intracomplex compounds, such as ethylenediaminotetra acetic and koyevaya ? acids. Ion surface active substances, apparently, physically block the active centers of ferments and hinder in this way the formation of ferment-substrate compounds (Fredrick,1957)

Group of vitamins with B_{12} factor (cobalamine) was found in Anabaena cylindrica in the amount of about 1 mg in 1 g of dry weight; this value is of the very same magnitude, as in bacteria, but higher, than in other algae and higher plants. Cyancobalamine constituted 65-70% of this amount, found were also traces of A and B factors (Brown, Cuthbertson, Fogg, 1956).

Separation of organic substances by bluish-green algae has been noticed repeatedly. In addition to amino acids and peptides, were detected separated Oscillatoria splendida cells of mucoid polysaccharides of oxalic, succinic, citric, tartaric acids, as well as volatile substances of the phytocyte type, representing aldehydes and volatile acids (Goryunova, 1950). Similar separations of certain representatives of bluish-green algae may exert an unfavorable effect on the quality of water, making it poisonous for fish and animals. Substances, separated by bluish-green algae in greater quantities, at points of their mass development, apparently, play a greater role in the formation of sapropela, dirt and humous soil complexes.

The nature of toxic substances separated by certain bluish green has been investigated very little. Toxic substances of filtrates of 5-6 week old cultures of Nostoc muscorum were found to be highly soluble in fatty solvents, but not in water. They had an alkaline reaction, fluoresced in ultraviolet light. But their nature is unknown (Jacob, 1957).

Polyphosphates were detected by Keck, Stich, 1957) in Phormidium sp and Oscillatoria sp, and Tischer, 1957, discovered same in 18 types of bluish-green algae.

Conclusion

It is evident from the presented review, that in recent years the number of investigations on the chemistry and biochemistry of bluish-green algae, has increased. This is due to a whole series of highly noticeable differences in the data of algae and other organisms. In first place attention is attracted by their noticeable ability to fix atmospheric nitrogen, in spite of the fact that the substances formed thereat are not specific only for bluish-green algae. Much remains unknown in the mechanism

of fixing the molecular nitrogen.

Among the additional pigments to chlorophyll an important is taken up by bilichromoproteines or phycobilines - phycoerythrin and phycocyanines. This brings them closer to red algae,and to the properties of carbohydrates as well.

The properties of bluish-green algae make them a quite interesting object for studying a whole series of general problems of biochemistry and plant physiology, the more so, since the separation of pure cultures from their natural populations can be realized relatively easily with the aid of certain antibiotics (Zehnder,Hughes 1958).

Although the total productivity of bluish-green is considerably lower than the productivity of such algae, as diatomic, in freash waters it may constitute a greater relative value and exert an influence on the biological productivity of this or any other reservoir. It should be pointed out that the photosynthetic ability of bluish-green is always lower than that of diatomeae (Tsyrina,1959).

The content of albumina in bluish-green algae reaches up to 35%, carbohydrates up to 70%, lipides up to 10%, ashes and other substances constitute 10-20% of dry weight. If it is assumed,that the amount of lipides in these algae equals 6% of the organic substance, then a 100 g of this substance will have 441 calories. But in spite of the quite greater calculated caloricity, a series of data allows to assume, that bluish-green algae do not have greater nutritional value for plankton animals and fish. In favor of this are speaking the results of chemical analyses (about 65% of organic substances constitute highly stable to hydrolysis carbohydrates), hydrobiological investigations and observations of water quality, of water which "blooms" with bluish-green algae.

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